Partial Depolymerization and Solubilization of Poly[(R)-3-hydroxybutanoate] (PHB) and Its Copolymer with (R)-3-Hydroxyvalerate (BIOPOL®) by Treatment with Li-Amides/LiCl in Tetrahydrofuran at Low Temperature [1]

Dieter Seebach*, Albert K. Beck, Urs Brändli, Dieter Müller, Michael Przybylski**, and Klaus Schneider**

Abstract. The biopolymers PHB and PHB/PHV (mol. weight > 7·10⁶ g·mol⁻¹) from fermentations of certain lot numbers or PHB samples which had previously been precipitated from dichloroethane solution can be dissolved by treatment with excess lithium-diisopropylamide in LiCl-containing tetrahydrofuran. The samples recovered from these solutions in good yield have molecular weights of 1000–5000 g·mol⁻¹.

A) Introduction

Polyhydroxybutyrate [2] (PHB) is a member of a class of biopolymers referred to as PHAs (polyhydroxyacids) which are produced by microorganisms. PHB and the copolymer containing up to 30% hydroxyvalerate units (PHB/PHV, BIOPOL®) are products made on an industrial scale [3] by fermentation of Hydrogenomonas eutropha (Alcaligenes eutrophus) [4]. Gene-technological methods have been applied to transfer the ability to produce PHB to other organisms [5] [6]. The high biocompatibility and the biodegradability of PHB and its congeners render these polymers attractive materials and have spurred expectations and speculations about future uses, as indicated by the title of a recent Science Research News Article, 'In Search of the Plastic Potato' [6]. The molecular weight of PHB from A. eutrophus is ca. 10³. Lower-molecular-weight oligomers of hydroxybutyrate could be useful, for instance as plasticizers for the high-molecular-weight material or for retard formulations in drug delivery. Besides degradation by depolymerases [7], mixtures of oligomers have been obtained previously by methanolysis [8] of PHB [9]. We have observed a surprising partial degradation of PHB (1 in Scheme 1) which was mentioned in a recent review article [10] and which is described in full detail herein.

B) Results

PHB of molecular weight of ca. 7.5·10⁶ (by light scattering; Mₘ) [11] was obtained from ICI Biological Products [12] as a sample of lot number MBL 100/80, a colourless, light fluffy material. It was dried, mixed with 3 equiv. LiCl and suspended in THF under an Ar atmosphere. The vigorously stirred mixture was cooled to temperatures between −75 and −105°C, and combined with a cold solution of 3 equiv. lithium diisopropylamide (LDA), 2,2,6,6-tetramethylpiperidide (LTMP), or hexamethyldisilazide (HMDS) in the same solvent (Table 1). The polymer dissolved and the mixture turned yellow. Quenching of this solution with aq. NaHCl caused a precipitate to be formed and the yellow colour to disappear. Evaporation of volatiles gave an aq. slurry from which samples of oligomeric hydroxybutyrate were extracted with CHCl₃ in yields ranging from 10 to 75%. PHB samples from other lot numbers, which were more dense, had to be precipitated by pouring a 1,2-dichloroethane solution into aq. MeOH to give the fluffy material (cf. runs number 2 and 4 in Table 2). The copolymer PHB/PHV (lot No. MBL 100/12, 22% PHV content) could likewise be dissolved in THF with a Li-amide base in the presence of LiCl. In both cases, in the absence of LiCl, an excess of up to 10 equiv. LDA had to be employed in order to achieve solution, conditions which were not further investigated.

The materials recovered from the THF solutions were investigated by gel-permeation chromatography (GPC), by osmometric methods (Mₘ, molecular weights), by differential scanning calorimetry (DSC), by ¹H-NMR [13a], and by plasma-desorption mass spectrometry (PDMS [13b,c], see Figs. 1-4 and Tables 1 and 2. Varying values for the molecular weight of a given sample were obtained by the different methods, but they all ranged from 1000 to 5000. We are not in a position to evaluate the reasons for these discrepancies at this stage, but we can safely make the following statements:

i) Except after prolonged periods of time, PHB (1) is not fully degraded to crotonate (2 in Scheme 1) by excess LiNR₃ in a LiCl-containing solution at low temperature.

ii) Oligomers 3a containing 10–50 hydroxybutyrate units with crotonate end groups are recovered from the THF solutions.

iii) The molecular-weight distribution in the mass spectrum of degraded PHB/PHV (Fig. 4) suggests that the hydroxyvalerate units are incorporated statistically in the bio-copolymer.

C) Discussion

Before hydrolysis, the THF solutions obtained from PHB and Li-amide bases must contain polyliithiated species which are associated with LiCl, excess LiNR₃, and HNR₃; see 4 in Scheme 2. It is the relatively large stability of these species towards elimination to crotonate (5 in Scheme 2) [14] and their solubility in the non-polar solvent THF, which are remarkable. The stability may be due to a highly complex structure containing intramolecular aggregations of Li-enolate moieties and intermolecular aggregations with the LiX species present (X = Cl or NR₃) [10], as well as complexation with the secondary amine, the by-product of deprotonation [10] [15]. The solubilization in THF of polyliithiated compounds by adding Li salts has also been observed with peptides [10] [16].

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The fact that the success of the partial depolymerization described here depends upon the source and pretreatment of the PHB samples (see Table 2) suggests that the molecular-weight range of the oligomers may not only be the result of special structural features in 4, but may also be caused by the presence of substructures in the PHB starting material (cf. the helical, crystalline domains of PHAs) [17]. Since there is access to open-chain [18] [19] and cyclic [18] [20] oligomers [7] [21] of 3-hydroxybutyric acid with defined chain lengths or ring size, we are now testing these possibilities.

We thank Dr. A. Rogg and A. Webb of the MBL Division, Billingham, England, for numerous helpful discussions and for carrying out some molecular-weight determinations of degraded PHB and PHB/PHV samples. Dr. S. Buntle and Y. Duchene of the Sandz AG, Basel, were generous enough to supply theGPC chromatograms and DSC curves for Figs. 1 and 2. The late Prof. P. Pino and M. Colussi, Institut für Polymerw., ETH Zürich, have been very helpful in introducing us to the techniques typical of polymer chemistry. T. Aeschbach's technical assistance in the large-scale degradation experiments and R. Breitschuh's experiments concerning acidic degradation are gratefully acknowledged. B. Lamatsch contributed the statistical calculations shown in Fig. 4. Support by the Deutsche Forschungsgemeinschaft is also gratefully acknowledged. R. Breitschuh's experiments concerning acidic degradation are gratefully acknowledged. B. Lamatsch contributed the statistical calculations shown in Fig. 4. Support by the Deutsche Forschungsgemeinschaft is also gratefully acknowledged. T. Aeschbach's technical assistance in the large-scale degradation experiments and R. Breitschuh's experiments concerning acidic degradation are gratefully acknowledged. B. Lamatsch contributed the statistical calculations shown in Fig. 4. Support by the Deutsche Forschungsgemeinschaft is also gratefully acknowledged.

Experimental

The molecular weights listed in Table 1 were determined by vapour-pressure osmometry using a Cornea Wescor 232 A/100 'Molecular Weight Apparatus' in CHCl₃ at 25°C. Calibration benzoic acid (M₀ = 201.2 g mol⁻¹) was used. The concentrations of the sample solns. measured were in the range 5–10 mg ml⁻¹. (i-Pr)(NH₂), hexamethyldisilazane (HMDS), and tetramethyldiamine (TMEDA) were distilled from CaH₂ or NaH prior to use. For small-scale reactions, THF was distilled from LiAIH₄ or K under Ar; for large-scale work, it was purchased from Fluka (p.a. grade).

Table 2. Preparative Degradation of PHB and PHB/22%/PHV on a 10-g Scale with Samples from Different Lots, Using LDA or LHMDS in THF at ~78°C. For details, see heading of Table I and procedure.

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Lot</th>
<th>Base LiNR₂</th>
<th>Yield [%]</th>
</tr>
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<tr>
<td>1</td>
<td>PHB-MBL 100/80</td>
<td>LHMDS</td>
<td>75%</td>
</tr>
<tr>
<td>2</td>
<td>PHB-MBL 100/393 (as received)</td>
<td>LHMDS</td>
<td>7%</td>
</tr>
<tr>
<td>3</td>
<td>PHB-MBL 100/393 (as received)</td>
<td>LDA</td>
<td>19%</td>
</tr>
<tr>
<td>4</td>
<td>PHB-MBL 100/393 (preheated)</td>
<td>LHMDS</td>
<td>51%</td>
</tr>
<tr>
<td>5</td>
<td>PHB/PHV-MBL 100/12</td>
<td>LDA</td>
<td>26%</td>
</tr>
<tr>
<td>6</td>
<td>PHB/PHV-MBL 100/12</td>
<td>LHMDS</td>
<td>58%</td>
</tr>
</tbody>
</table>

*) GPC and DSC see Figs. 1 and 2. b) By addition of CICH₂CH₂CI solution to MeOH/H₂O, see accompanying text and Experimental. c) PDM, see Fig. 3 (B).

Scheme 1

Scheme 2

Table 1. Degradation of PHB (lot MBL 100/80, 1-kg scale batch) with LiNR₂ in THF with Different Reaction Times and at Different Temperatures. The reaction mixtures were quenched by injecting excess sat. aq. NH₄Cl into the stirred cold soln.; the molecular weights (M₀) were determined by vapour-pressure osmometry (see Experimental and accompanying text). In all runs, 3 equiv. LiCl and LiNR₂ per hydroxybutanoate unit were employed. Abbreviations for the bases, see text. Differential scanning calorimetry (DSC) of some samples with M₀ = 2100 – 3000 gave m.p. (max. peak temp.) between 128 and 140°C, ranges of melting from 115 to 155°C, and enthalpies of melting from 5700–7000 J·mol⁻¹; see also Figs. 2. Estimation of the sample of M₀ = 2737 with CH₃N₂ and ²H-NMR-spectroscopic analysis of the C–CH₂O–CH₂ ratio leads to a molecular weight of 2450.)

<table>
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<tr>
<th>Base LHMDS</th>
<th>Reaction time [min]</th>
<th>Temperature [°C]</th>
<th>Yield of oligomer [°C]</th>
<th>M₀ [g mol⁻¹]</th>
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<td>2935</td>
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<td>2737</td>
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<tr>
<td>LDA 8</td>
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<td>-105</td>
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<td>3060</td>
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a) The crotonate C=C–CH₂ signal appears at 1.85 ppm (dd, J = 7.5,1) (CDCl₃, 200 MHz, Varian-Gemini spectrometer) [13a].

b) The yield in this run is somewhat smaller because the sample was isolated by taking an aliquot out of the reaction mixture, transferring it to another flask (~78°C) and quenching.

c) Quenching after 15 h causes no more precipitation of oligomers. According to NMR analysis of the acid fractions from the workup procedure, crotonic acid and its non-conjugated isomer, but-3-enoic acid are the products of this ‘total’ degradation.

The reaction mixtures were quenched by injecting excess sat. aq. NH₄Cl into the stirred cold soln.; the molecular weights (M₀) were determined by vapour-pressure osmometry (see Experimental and accompanying text). In all runs, 3 equiv. LiCl and LiNR₂ per hydroxybutanoate unit were employed. Abbreviations for the bases, see text. Differential scanning calorimetry (DSC) of some samples with M₀ = 2100 – 3000 gave m.p. (max. peak temp.) between 128 and 140°C, ranges of melting from 115 to 155°C, and enthalpies of melting from 5700–7000 J·mol⁻¹; see also Figs. 2. Estimation of the sample of M₀ = 2737 with CH₃N₂ and ²H-NMR-spectroscopic analysis of the C–CH₂O–CH₂ ratio leads to a molecular weight of 2450.)

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Reprecipitation of PHB

PHB lot No. MBL 100/80 (50 g) was taken up in 1,2-dichloroethane (250 ml) to afford a nearly clear, brownish soln. This was heated under reflux and CH₂Cl₂ (750 ml) was added over 10 min. After cooling to r.t., the soln. was filtered through a 3-cm pad of Celite (diameter 6.5 cm) under suction. The Celite was washed with CH₂Cl₂ (100 ml). The resulting, almost colourless, clear soln. was concentrated to a volume of 500 ml and then poured into a vigorously stirred mixture of MeOH (1500 ml) and H₂O (600 ml) [24]. The fluffy white material which precipitated was filtered off and dried under high vacuum over P₂O₅ to constant weight (48.5 g).

Partial Depolymerization of PHB and PHB/PHV

Small scale (PHB)

THF (100 ml) was syringed into a 2-necked flask containing PHB (1 g, 12.6 mmol) and LiCl (1.48 g, 34.8 mmol) under Ar. The mixture was stirred for 1 h to enable the PHB to swell up and then cooled to −78°. A cold (−78°) soln. of LDA (3 equiv.) in THF (13 ml), generated from DIPA (5.3 ml, 37.2 mmol) and BuLi (26 ml, 40.5 mmol), was added via syringe to give a clear, yellow soln.

After different periods of time (see Table 1), the reaction was quenched by addition of sat. aq. NH₄Cl soln. (10 ml) via syringe to give a precipitate. The mixture was allowed to warm up to r.t. and the THF evaporated. The residue was taken up in CH₂Cl₂ and the org. layer washed with sat NaHCO₃ soln. (2 x 50 ml), 1N HCl (2 x 150 ml), and brine (2 x 150 ml), dried (MgSO₄), and concentrated. Pentane or Et₂O was then added to precipitate the degraded PHB.

For the reaction of PHB with LHMD and LTMP, one half and one third of the above quantities were used, respectively.

Large scale (PHB)

A mixture of PHB (10 g, 126 mmol) and LiCl (16 g, 348 mmol) in THF (1 l) in a 24-necked flask was stirred at r.t. for 2 h and then cooled to −75° (internal temp.) [25]. LHMD (3 equiv.) was generated from HMDS (75 ml, 360 mmol) and BuLi (240 ml, 362 mmol) in THF (100 ml) and added as cold (−75°, internal temp.) solid/liquid mixture via a Teflon cannula (diameter 2.5 mm) [26]. A further 20 ml THF was used to rinse the flask used for generation of the amide base, and this was also added to the reaction vessel.
Fig. 4. Comparison of measured and calculated molecular-weight distribution in degraded PHB/22% PHV. (A) PDMS signals between 1550 and 1750 Daltons (B) Ratio of masses calculated for a fragment containing 18 and 19 \( \beta \)-hydroxy-acid units, with the assumption that the valerate moieties are statistically distributed.

The resulting mixture was difficult to stir and was removed briefly from the cooling bath and shaken by hand to afford a pale yellow, turbid soln. This was then stirred for 15 min at which time the internal temp. was \(-68^\circ\) Sat. NH\(_4\)Cl soln. (100 ml) was added by syringe, whereupon the temp. of the mixture became \(-38^\circ\), and a white solid precipitated. The mixture was then stirred at r.t. for 2 h. The THF was evaporated and the residue taken up in CHCl\(_3\) (800 ml) and washed with half-sat. NaHCO\(_3\) soln. (600 ml) [24]. The aq. phase was re-extracted with CHCl\(_3\) (200 ml), and the combined org. layers were washed with sat. NaHCO\(_3\) soln. (2 x 300 ml). During this washing procedure, a gelatinous substance formed at the CHCl\(_3\)/H\(_2\)O interface which was removed by filtering the mixture through Celite. The org. portion of the filtrate was washed with H\(_2\)O (500 ml), dried (MgSO\(_4\)), and evaporated to furnish 9.3 g of a beige-coloured solid. This was stirred with Et\(_2\)O (100 ml) at r.t. for 2 h and the solid then collected on a frit. Drying under high vacuum afforded 7.5 g of a white powder.

For runs 2 and 3 (Table 2) substantial amounts of solid could be filtered off from the aq. phase after the first extraction with CHCl\(_3\). This material was presumably non-degraded PHB. Run 3 was carried out using the above general procedure but with disopropylamine (DIPA) instead of HMDS as the amine component.

Fig. 3. Plasma desorption mass spectra (PDMS) of partially degraded PHB (A) and PHB/22% PHV (B). 5-30 \( \mu \)g in CHCl\(_3\) electrosprayed on nitrocellulose. Spectra obtained on BIO-ION 20 \( k \) instrument, 15 kV accelerating voltage, start counts A) 3 \( \cdot \) 10\(^5\), B) 10 \( \cdot \) 10\(^5\). No peaks were detected in the same molecular-weight range, when the non-degraded polymer was applied. The PHB sample was from run 4 of Table 1, the PHB/22% PHV sample from run 5 of Table 2. Number of PHB residues are indicated (A), for PHB/22% PHV residues (B) see Fig. 4.
Large scale (PHB/22% PHV)
The transformations were carried out according to the general procedure described above for PHB.

Received: February 9, 1990

[8] Heating PHB in 1,2-dichoroethane with less than equimolar amounts of MeOH in the presence of T3OH also leads to mixtures of oligomers with methyl-ester end groups (cf. partial depolymerisations by BF3·OEt2/MeOH: S. Coulombe, P. Schauwecker, R. H. Marchessault, B. Häfette- cough, Macromolecules 1978, 11, 279). The average molecular weight of these oligomers can be determined by NMR analysis (ratio of C-CH2 to O-CH2 intensity). Depending upon the ratio PHB/MeOH oligomers of different chain lengths are obtained. Thus, after 48 h of reaction time with 1.0, 0.5, 0.2, 0.1 equiv. MeOH, samples were isolated which had average chain lengths of 1.4, 2.1, 5.0, 10.0, resp. A sample withdrawn from the reaction mixture set up using 0.5 equiv. MeOH after 4 h exhibited the following plasma desorption mass spectrum. It is interesting to note that the mass peak intensities alternate in such alternations of properties with chain lengths are quite common: m.p. of hydrocarbons (see textbooks of organic chemistry), cyclizations to medium-size rings [Y. Prelog, J. Chern. Soc. 1950, 420], second order asymmetric transformations [K. Weinges, B. Stemmle, Recent Developments in the Chemistry of Natural Carbon Compounds 1976, 7, 89].
[12] We thank MBL Division, Bellingham, Great Britain, for PHB and BIOPOU.
[14] There is no indication in the NMR spectra of the recovered samples that the polyester chain was cleaved by nucleophilic attack of lithium amide on the ester carbonyl group (no RCO-N(CHMe2)2 signals, when LiDA was used).
[24] The distribution/precipitation of PHB between aq. or aq/alkoholic and CH2Cl2 or CHCl3 solns. is a commonly used procedure for purification, see e.g. a recent paper on synthetic PHB obtained by stereoselective polymerisation of rac-3-hydroxybutyra- tone with Lewis acids in the presence of (R)-3,3- dimethylbutane-1,2-diol: A. Le Borgne, N. Spagnys, Polymer 1989, 30, 2312.
[25] A Pt 100 thermometer with digital recorder for measurement of the temper. was used.

Nomenclature of Organic Polycycles out of the Computer – How to Escape the Jungle of the Secondary Bridges

Gerta Rücker and Christoph Rücker

Abstract. A computer program is described which generates IUPAC names (von Baeyer names) and the corresponding numbering schemes for polycyclic hydrocarbons of any size and complexity. The program thoroughly uses constitutional symmetry which may be present. Parts of IUPAC rule A-32 had to be formulated more precisely than codified hitherto. The names and the elapsed CPU times are given for some polycycles.

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